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Association of maternal serum PAPP-A levels, nuchal translucency and crown rump length in first trimester with adverse pregnancy outcomes: Retrospective cohort study

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1 **Association of maternal serum PAPP-A levels, nuchal translucency and crown rump length**
2 **in first trimester with adverse pregnancy outcomes: Retrospective cohort study**

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33 There was no specific funding for this study.

34

35 **Disclosure of interests**

36 Dr Morris is an author of the RCOG Greentop guideline on Investigation and Management of
37 the Small for Gestational Age Fetus.

38

39 **What's already known on this topic?**

40 Low levels of PAPP-A are associated with small for gestational age and pre-eclampsia.

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42 **What does this study add?**

43 NT, CRL and PAPP-A are independent prognostic markers for adverse pregnancy outcome.

44 Further work is required to assess the predictive ability of these factors in prediction

45 models.

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47 **Abstract**

48 **Objective**

49 Are first trimester serum pregnancy-associated plasma protein-A (PAPP-A), nuchal
50 translucency (NT) and crown rump length (CRL) prognostic factors for adverse pregnancy
51 outcomes?

52 **Method**

53 Retrospective cohort women, singleton pregnancies (UK 2011-2015). Unadjusted and
54 multivariable logistic regression, outcomes: small for gestational age (SGA), pre-eclampsia
55 (PE), pre-term birth (PTB), miscarriage, stillbirth, perinatal mortality and neonatal death
56 (NND).

57 **Results**

58 12,592 pregnancies: 852 (6.8%) PTB, 352 (2.8%) PE, 1824 (14.5%) SGA, 73 (0.6%)
59 miscarriages, 37(0.3%) stillbirths, 73 perinatal deaths (0.6%) and 38 (0.30%) NND.
60 Multivariable analysis: lower odds of SGA [adjusted odds ratio (aOR) 0.88 (95% CI
61 0.85,0.91)], PTB [0.92 (95%CI 0.88,0.97)], PE [0.91 (95% CI 0.85,0.97)] and stillbirth [0.71
62 (95% CI 0.52,0.98)] as PAPP-A increases. Lower odds of SGA [aOR 0.79 (95% CI 0.70,0.89)]
63 but higher odds of miscarriage [aOR 1.75 95% CI (1.12,2.72)] as NT increases, and lower
64 odds of stillbirth as CRL increases [aOR 0.94 95% CI (0.89,0.99)]. Multivariable analysis of
65 three factors together demonstrated strong associations: a) PAPP-A, NT, CRL and SGA, b)
66 PAPP-A and PTB, c) PAPP-A, CRL and PE, d) NT and miscarriage.

67 **Conclusions**

68 PAPP-A, NT and CRL independent prognostic factor for adverse pregnancy outcomes,
69 especially PAPP-A and SGA with lower PAPP-A associated with increased risk.

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72 **Keywords:** PAPP-A, small for gestational age, stillbirth, pre-eclampsia, pre-term delivery.

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74 **Introduction**

75 Adverse pregnancy outcomes have a psychological impact for the family as well as
76 an increased cost of healthcare. Methods of prediction would allow obstetricians to provide
77 increased obstetric surveillance, focusing optimum management and possibly improving the
78 outcome of the pregnancy.

79 Pregnancy associated plasma protein A (PAPP-A) is a placental glycoprotein
80 produced by syncytial trophoblast of the placenta, which cleaves insulin-like growth factor
81 binding protein 4 (IGFBP4) and is a positive regulator of insulin-like growth factors (IGFs) ¹,
82 potentially influencing fetal growth and wellbeing.

83 Studies have tested the hypothesis that low maternal serum levels of PAPP-A in the
84 first trimester are prognostic factors for adverse pregnancy outcomes associated with poor
85 placental function ^{2 3, 4 5 6}. International Guidelines on “*The Investigation and Management*
86 *of the Small for Gestational Fetus*” have recommended that pregnant women with a serum
87 PAPP-A <0.4MoM (5th centile) in the first trimester receive increased ultrasound
88 surveillance for fetal growth disorders⁷. This recommendation was based on a previous
89 systematic review by our group in 2008 assessing Down’s syndrome markers to predict pre-
90 eclampsia and SGA⁸. This review included only 16 studies, did not assess all outcomes and
91 did not distinguish between prognosis and prediction. However, contradictory results have
92 been observed in publications ^{6, 9} and few studies have investigated the association of first
93 trimester fetal biometry [nuchal translucency (NT) and crown rump length (CRL)] with
94 adverse outcomes and their relationship with PAPP-A ^{3, 10}. Resolution of this is required,
95 because genuine prognostic factors in this field have many potential uses. As outlined by the
96 PROGRESS series ¹¹⁻¹⁴, they may guide clinical decisions and monitoring strategies, inform

the design and analysis of new trials, and improve models for individualised risk prediction. Factors that add additional (independent) prognostic information are difficult to find, but are needed to improve the discrimination performance of a 'prognostic model'¹³ that produces absolute risk predictions for women based on a set of individual characteristics¹¹. Our objective was to undertake a large cohort study to determine whether serum PAPP-A, NT and CRL in the first trimester are independent prognostic factors for the risk of subsequent adverse pregnancy outcomes.

Methods

Data collection

In a retrospective cohort study, data were collected from patients booked from 1st August 2011 (commencement of electronic maternity record) to 31st March 2015 at the Birmingham Women's Foundation Trust (BWNFT), a secondary and tertiary care NHS hospital in West Midlands, UK.

All pregnant women who accepted first trimester aneuploidy screening and delivered in BWNFT were included in the study. First trimester aneuploidy screening is offered to all pregnant women between 11+2 to 14+1 weeks of gestation (crown-rump length (CRL) measures from 45 mm to 84 mm) as part of the National Down Syndrome Screening Programme^{15, 16}. This involves measuring maternal serum levels of PAPP-A and free beta human chorionic gonadotrophin (fβHCG), along with the NT, and the pregnancy is dated on the basis of CRL. All first trimester scans and measurements performed at BWNFT are performed by accredited sonographers according to National NEQAS guidelines¹⁷. First

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119 day of the last menstrual period was obtained from the referring letter from the community
120 midwife or hospital. This date was confirmed with the mother at the ultrasound visit and
121 additional information on the regularity and cycle duration was obtained.

122 Analysis for PAPP-A was performed on the Auto-Delfia immunoassay platform
123 (Perkin Elmer Ltd, Seer Green, UK). For the purpose of Down’s syndrome screening, PAPP-A
124 values are converted to multiples of the median for gestation. In this study, to prevent any
125 loss of data¹⁸ and to remove the need for considering an absolute threshold, PAPP-A values
126 were considered as a continuous variable using the absolute value (mU/L). To aid the
127 presentation and interpretation of results, PAPP-A values were rescaled by dividing the
128 values by 1000, therefore the results relate to PAPP-A values in U/L.

129 Multiple hospital-based, secure and confidential computerized databases were used
130 to extract the information for the study and governance from the hospital-based IT and
131 Caldecott Guardian were prospectively obtained. Maternal and pregnancy outcome data
132 were collected from K2 database, Neonatal data were collected from Badgernet database,
133 and biochemistry data were collected from a biochemistry database. The collected data
134 consisted of maternal characteristics and demographics; maternal medical, antepartum,
135 peripartum and pregnancy outcome data along with first trimester serum PAPP-A levels, NT
136 and CRL. Multiple gestations, pregnancies with donor eggs, missing outcome and known
137 fetal aneuploidy were excluded. Cases of aneuploidy were cross referenced with the West
138 Midlands Regional Genetics Laboratory database.

139 **Definitions of maternal and obstetric characteristics**

Preterm birth (PTB) was defined as live delivery prior to 37 weeks, both spontaneous and iatrogenic. Pre-eclampsia (PE) was defined according to the International Society for the study of Hypertension in Pregnancy (ISSHP) definition as *de-novo* hypertension at or after 20 weeks gestation (at least 2 readings of Blood Pressure >140 mmHg systolic or >90 mmHg diastolic) with proteinuria (spot urine protein/creatinine >30 mg/mmol [0.3 mg/mg] or >300 mg/day or at least 1 g/L ['2 + '] on dipstick testing)¹⁹. Small for gestational age (SGA) was defined as birthweight below the 10th percentile of the customised growth chart²⁰. Miscarriage was defined as fetal demise before 24 weeks of gestation. Stillbirth was defined as intrauterine death after 24 completed weeks of pregnancy. Perinatal death was defined as fetal or neonatal death between 24 weeks of gestation and 7 days after birth. Neonatal death was defined as death between birth and 28 days.

Data Analysis

Mother and fetus demographics and clinical features

Distributions of demographic characteristics and known prognostic factors were summarised. The existing prognostic factors were those deemed to be important by the clinical team a prior and defined as: maternal age at test, gestational age at test, parity, body mass index (BMI), deprivation category (Index of Multiple Deprivation 2010 – IMD¹⁶, calculated using National Perinatal Epidemiology Unit calculator), ethnicity, assisted conception (IVF), smoking status (non-smoker, stopped at booking or on-going smoker), pre-pregnancy insulin-dependent diabetes mellitus, and gender of the baby. Mean and standard deviation (SD), or median and interquartile range (IQR), is reported for continuous variables,

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161 according to whether the variables were normally distributed. The number and percentage
162 are reported for categorical variables.

163 *Analysis of prognostic association with outcomes*

164 Univariable logistic regression analysis was used to estimate the unadjusted odds
165 ratio (OR) for each potential prognostic factor (PAPP-A/NT/CRL) separately. The odds ratios
166 indicate how much the odds of the outcome are increased for each 1-unit increase in the
167 factor.

168 Again for each of the three factors separately, multivariable logistic regression
169 analyses were fitted to examine the odds ratio adjusted for the known (or likely) existing
170 prognostic factors of maternal age (years), parity, BMI, smoking status, IVF, ethnicity,
171 deprivation category and gestational diabetes. This provided the adjusted odds ratio for a 1-
172 unit increase in each factor, to reveal their independent prognostic value over and above
173 other factors.

174 Then, for each outcome, the three factors were analysed in combination in one
175 multivariable logistic regression model, whilst adjusting for the other factors detailed above,
176 to explore whether the prognostic value of each factor is the same after adjusting for the
177 other two potential prognostic factors. The linearity assumption of all continuous variables
178 was assumed for these multivariable models.

179 Therefore, finally, the fully adjusted models with all three potential factors in
180 combination were fitted again; however, additionally, the linearity assumption of the
181 prognostic effects for the three factors of interest was assessed, and alternative functional
182 forms were considered if the assumption was violated. Else, a linear relationship was

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3 183 specified for all three prognostic factors of interest. The functional form was chosen using
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5 184 fractional polynomials, where all possible fractional polynomials up to the second degree
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7 185 were considered based on their statistical significance²¹. A linear relationship was specified
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10 186 for the other continuous covariates (maternal age and BMI).

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13 187 In all multivariable models described above, no model selection process was used to
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15 188 determine which factors were included in each model, since all variables were pre-specified.

16 189 *Handling of missing data*

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22 190 The percentages of missing values for each covariate and outcome were calculated.
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24 191 Missing data was imputed with multiple imputation with chained equations with 35
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26 192 imputed datasets equal to the percentage of patients with missing data²². For non-normally
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28 193 distributed variables, predictive mean matching was used to impute the missing data²³. The
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30 194 imputation model contained all complete outcomes and covariates that were included in
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32 195 the multivariable analyses. Rubin's rules were used to combine the parameter estimates
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34 196 and standard errors into a single inference²⁴. A complete-case analysis was also conducted
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36 197 as a sensitivity analysis.

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41 198 Estimates of prognostic effects are reported as odds ratios (OR) with 95% confidence
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43 199 intervals (CI) and p-values. Analyses were performed using Stata version 14²⁵ and all
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45 200 regression models were fitted using maximum likelihood estimation.

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50 202 **Results**

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203 Between August 2011 and March 2015, first-trimester combined screening was
204 performed in 12,837 pregnant women. Ten pregnancies conceived with donor eggs were
205 excluded. Three pregnancies with an unclear pregnancy outcome showing neonatal death
206 post 28 days were excluded since this outcome would not be routinely recorded in the
207 databases. After excluding 232 multiple pregnancies, the final study cohort was of 12,592
208 singleton pregnancies.

209 As depicted in Table 1, the mean maternal age and median BMI were 30.6 (SD 5.6)
210 and 25.1 (IQR 22.4 to 28.8), respectively. The majority of the women were White British
211 European (62.6%) followed by South Asian (19.9%). Mean gestational age at first trimester
212 ultrasound was 88.2 days (SD 4.3). About 2% (n=250) of pregnancies were the result of
213 assisted conception. Smoking status was known for all women and 12.4% were on-going
214 smokers in the pregnancy, 84.3% non-smokers and 3.3% had given up at the time of first
215 trimester booking appointment. Nearly half (43%, n=5495) patients lived in the most
216 deprived areas (deprivation score \geq 34.18) and 3.6% (n=455) patients lived in the least
217 deprived areas (deprivation score \leq 8.49). Gender distribution was almost equal in the
218 fetuses (48.6% male and 51.1% female).

219 BMI was missing in 4466 (35.5%) records, parity was missing in 567 (4.5%) and
220 deprivation score was missing in 175 (1.4%) records. There were missing data for 152 (1.2%)
221 fetal weight and four (0.03 %) neonatal outcomes. Multiple imputation was performed for
222 missing BMI (height, weight), parity and deprivation score values. Although some variables
223 were normally distributed, predictive mean matching was used for all variables because
224 imputation with chained regression analysis imputed unrealistic values for weight and
225 height.

Table 2 displays the number of events for each outcome for the cohort, and the prognostic association of PAPP-A, NT and CRL with these outcomes in the unadjusted analyses. Of 12,592 women, 852 had pre term birth (6.8%), 352 patients had pre-eclampsia (2.8%) and 1824 babies were SGA (14.5%). There were 73 pregnancies that ended in miscarriage (0.6%) and 37 stillbirths (0.3%). There were 38 neonatal deaths (0.31%) of which 36 were early and thus giving a total of 73 perinatal deaths (0.6%).

PAPP-A Results

In the unadjusted analysis (Table 2), a one unit increase in the concentration of PAPP-A (U/L) was estimated to lower the odds of an SGA neonate by 13%, which was highly significant [OR 0.87 (95% CI 0.85, 0.90), $p < 0.0001$]. Similar conclusions can be drawn for the association between PAPP-A and pre-term birth [OR 0.93 (95%CI 0.90, 0.97), $p < 0.0001$] In addition, PAPP-A and pre-eclampsia demonstrated a similar relationship [OR 0.92 (95% CI 0.86, 0.97), $p = 0.004$]. The results for stillbirth were in the same direction and quantitatively similar, although the CI was slightly wider and the p-value was just above the 5% [OR 0.81 (95% CI 0.66, 1.0), $p = 0.052$]. There was no evidence of a strong association between PAPP-A and miscarriage, perinatal death or neonatal death.

After adjusting for mother's age, BMI, parity, ethnicity, deprivation score, smoking status, IVF status, and gestational diabetes in the multivariable analysis there was evidence of strong independent prognostic associations between: PAPP-A and SGA [OR 0.88 (95%CI 0.85, 0.91), $p < 0.0001$] (Table 3; full model parameter estimates for all adjusted models are shown in the Supporting Information Tables S1-S7); PAPP-A and PTB [OR 0.92 (95% CI 0.88, 0.97), $p < 0.0001$]; and PAPP-A and PE [OR 0.91 (95% CI 0.85, 0.97), $p = 0.003$]. There was also

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evidence of a lower odds of a stillbirth as PAPP-A increases [OR 0.71 (95% CI 0.52, 0.98),
p=0.038]. The association of PAPP-A with the outcomes of miscarriage (Table S4), perinatal
death (Table S6) and neonatal death (Table S7) remained non-significant in the multivariable
analyses.

NT Results

In the unadjusted analyses (Table 2), for higher values of NT there was a strong
association with an increased odds of miscarriage [OR 1.94 (95% CI 1.54, 2.45), p<0.0001],
and a significant decreased odds of SGA [OR 0.81 (95% CI 0.72, 0.91), p<0.0001]. There was
also some evidence that higher values are associated with an increased risk of PTB [OR 1.15
(95% CI 1.00, 1.32), p=0.053] though the CI overlapped one.

After multivariable analysis, there was independent prognostic value of NT for SGA
[OR 0.79 (95% CI 0.70, 0.89), p<0.0001] (Table 3 and Table S8), and for miscarriage [OR 1.75
(95% CI 1.12, 2.72), p=0.013] (Table S11). There was no significant relationship between NT
and PTB, PE, stillbirth, perinatal or neonatal death in the unadjusted or adjusted analyses
(Table S9, S10, S12, S13, S14).

CRL Results

For CRL in the unadjusted analysis there was no significant association with any of
the outcomes (Table 2). There was a borderline statistical significance for SGA which
remained after adjustment [OR 0.99 (95% CI 0.99, 1.00, p=0.057)] (Table 3), but the
magnitude of the OR was close to one. After adjustment for other known predictors and
potential confounders, there was evidence of a strong association between CRL and
stillbirth [OR 0.94 (95% CI 0.89, 0.99), p=0.027], thus between 1% and 11% lower odds of

stillbirth for a one unit increase in CRL. The adjusted analyses for PTB, PE, miscarriage, perinatal death and neonatal death demonstrated no significant association with CRL (Table S15 - S21).

PAPP-A, NT and CRL in combination

Assuming linear functions for all continuous variables, the three potential prognostic factors were then considered in combination with adjustment for confounders and known prognostic factors as discussed (Tables S22-28 in the Supporting Information). For SGA, this analysis demonstrated strong associations with PAPP-A [OR 0.87 (95% CI 0.84, 0.90), $p < 0.0001$]; NT [OR 0.80 (95% CI 0.70, 0.91); $p = 0.001$] and CRL [OR 1.01 (95% CI 1.00, 1.03); $p = 0.004$].

For preterm birth (Table S23), only PAPP-A was significantly associated with reduced odds [OR 0.92 (95% CI 0.87, 0.96), $p < 0.0001$], as seen when the factors were considered individually. For pre-eclampsia, PAPP-A was still significantly associated [OR 0.88 (95% CI 0.82, 0.94); $p < 0.0001$] and now there was evidence of increased odds of pre-eclampsia as CRL increases [OR 1.02 (95% CI 1.01, 1.04); $p = 0.004$] (Table S24).

There remained a statistically significantly increased odds of miscarriage as NT increases [OR 1.67 (95% CI 1.01, 2.76), $p = 0.047$], and no evidence of associations between miscarriage and PAPP-A or CRL (Table S25). There was not any strong evidence (e.g. based on statistical significance at the 5% level) of any associations between stillbirth and any of PAPP-A, NT or CRL, unlike the individual models (Table S26). There was no evidence of associations between any of the three factors of interest and perinatal or neonatal death (Table S27-28).

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293 After checking the linearity assumption of the three prognostic factors, all the
294 previously identified prognostic associations remained the same. However, the log
295 transformation was statistically the best fitting functional form of PAPP-A for SGA, and
296 $1/\sqrt{\text{PAPP-A}}$ was statistically the best fitting functional form of PAPP-A for PTB. For PAPP-A for
297 all other outcomes and for CRL and NT for all outcomes, the best fitting functional form was
298 the linear function (See Tables S29-S31).

299 All the findings remained the same in the complete case analysis.

300 **Discussion**

301 **Main Findings**

302 This large cohort study provides strong evidence that lower values of PAPP-A are
303 associated with an increased odds of SGA, stillbirth, PE and PTB. As NT increases there is
304 evidence of a lower odds of SGA but higher odds of miscarriage. As CRL decreases there is
305 evidence of higher odds of stillbirth. Neonatal and perinatal deaths were not associated
306 with any of the prognostic factors measured in the first trimester. When considered in
307 combination there is a statistically significant association of PAPP-A, NT and CRL with SGA;
308 preterm birth with PAPP-A, pre-eclampsia with PAPP-A and CRL, and miscarriage with NT. In
309 the combined model stillbirth is no longer associated with any of the factors.

311 **Strengths and Limitations:**

312 Our study has several strengths. This is a large cohort study looking at multiple
313 pregnancy outcomes providing reproducible statistical results. The UK is a country where
314 high quality and homogenous universal health care is provided to its residents free of charge

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3 315 irrespective of socioeconomic and other statuses. This made our cohort a true
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5 316 representative of the general population avoiding bias due to skewed demographics.
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7 317 Certain factors that are known to affect pregnancy outcome, such as ethnicity, parity,
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9 318 maternal age and BMI, socio economic deprivation, smoking status and pre-pregnancy
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11 319 insulin-dependent diabetes mellitus, have been adjusted for in our analysis. We have made
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13 320 an effort to look for lesser researched possible associations such as miscarriage and
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15 321 neonatal/perinatal death. Despite these strengths, our study is not without limitations. The
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17 322 data for potential confounding factors (existing prognostic factors) was limited to that which
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19 323 is routinely collected in our electronic maternity record as this was a retrospective study.
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22 324 Similarly, the outcomes are limited to those routinely recorded and thus it was not possible
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24 to look at different thresholds that might confer a more severe outcome e.g. birth weight
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26 <5th or 3rd customised centile or severe pre-eclampsia. However, as our study was designed
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28 to investigate the independent prognostic ability of first trimester factors with adverse
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30 outcome it can be argued that when a factor is prognostic this relationship will be stronger
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32 for higher thresholds for the same outcome. Although the databases used were not
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34 329 designed for this particular study they are populated by qualified health professionals and
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36 330 data was obtained from multiple sources to allow cross-referencing and checking of
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38 331 outcomes. The biochemistry data is part of the National Screening Programme and thus
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40 332 subject to the relevant quality assurance (UK National external quality assurance scheme
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42 333 (UKNEQAS), Edinburgh Royal Infirmary, UK and Downs syndrome screening quality
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44 334 assurance and support service (DQASS), University of Plymouth UK). Our sample was
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46 335 determined by the number of patients available with an electronic record and outcome data
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48 336 and thus not determined by a sample size calculation. Most confidence intervals were quite
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3 338 narrow, but we recognise that non-significant findings do not necessarily mean that no
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5 339 prognostic association exists, and may simply reflect a low power to detect genuine
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8 340 associations. Nevertheless, many confidence intervals were relatively narrow and the
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10 341 prognostic associations identified were often strongly significant ²⁶.
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12 342 The clinical utilisation of CRL as an individual prognostic factor (i.e. outside of a model using
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14 343 it as a continuous factor) is less clear as standard care in the UK is for a single first trimester
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17 344 ultrasound to incorporate dating (using CRL) and NT for aneuploidy risk. Previous studies
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19 345 have assessed the prognostic value of difference in expected to observed CRL based on the
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21 346 last menstrual period¹⁰, observed versus expected change in CRL in the first trimester²⁷ and
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23 347 CRL as a continuous factor in multivariable analysis²⁸. The use of CRL to date a pregnancy
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26 348 assumes that there is no growth variation within the first trimester nor association with
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29 349 factors such as fetal sex, maternal age or ethnicity²⁹. A study from the Netherlands
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31 350 demonstrated that CRL in the first trimester was associated with an increased risk of
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33 351 adverse birth outcomes and postnatal growth acceleration²⁸. As standard care in the UK is
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36 352 only to offer one first trimester ultrasound, thus it is not possible to assess CRL change and
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38 353 the use of the CRL to date the pregnancy does not allow the assessment of observed to
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41 354 expected CRL. We thus wished to assess whether CRL, measured between the 11+2-14+1
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43 355 week window, assessed as a continuous variable had a relationship with adverse pregnancy
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45 356 outcome i.e. in particular assessing extremes of the continuum.

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52 358 **Interpretation**

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3 359 The aim was to provide more evidence toward establishing if the prognostic factors of
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5 360 interest can be used to further inform the management of potential adverse outcomes, for
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8 361 example by increased surveillance for pregnant women at greater risk. The results showed
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10 362 evidence of associations between the potential prognostic factors and several outcomes,
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12 363 and the associations remained largely the same when the factors were considered in
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14 364 combination. Future work is now important to establish whether the findings from all
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16 365 prognostic factor studies are consistent by synthesising the evidence.
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20 366 The evidence from this study supports the need for women with pregnancies with a low
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22 367 PAPP-A and increased NT being under Consultant led care and the recommendation within
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24 368 the RCOG guidelines⁷ for increased surveillance for SGA in pregnancies with a low PAPP-A
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26 369 and supports this being extended to pregnancies in the first trimester with an increased NT.
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29 370 At present until a model is developed that can incorporate these factors as continuous
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31 371 variables it would be appropriate to use accepted thresholds of <5th centile for PAPP-A and
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33 372 >99th centile for NT. Due to the association with low PAPP-A and PTB and PE these
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35 373 pregnancies should be assessed comprehensively for other risk factors for PTB and
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37 374 consideration given to the commencement of aspirin prior to 16 weeks. Independent
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39 375 prognostic factors have a broad array of potential uses in both clinical practice and health
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41 376 research¹². For instance, they help to define disease at diagnosis; they may be modifiable in
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43 377 order for interventions to improve outcomes; they aid the design and analysis of trials; they
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45 378 are confounders to consider in observational studies and unbalanced trials; and they are the
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47 379 building blocks of prognostic models¹². Prognostic factor research is therefore important to
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49 380 discover and evaluate such factors.
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381 We emphasise that our multivariable models were fitted to examine if there is evidence of
382 an independent association between the potential prognostic factors of interest and the
383 maternal and fetal outcomes after adjustment for known prognostic factors. Our objective
384 was to assess the prognostic factors themselves and not on an overall prognostic model for
385 individual risk prediction. This is especially important since there was no external data to
386 validate such a model¹³. Future work could use new datasets to develop individual risk
387 prediction models in order to tailor treatment choices to the individual and to look at
388 different thresholds for the outcomes e.g. severe PET. Such models should build on the
389 findings of this study, in terms of the prognostic factors that were identified as important.

390 **Conclusion**

391 When three first trimester potential prognostic factors are considered in combination there
392 remains strong evidence of associations between: a) PAPP-A, NT, CRL and SGA, b) PAPP-A
393 only with PTB, c) PAPP-A and CRL for PE, d) NT and miscarriage. Further work is required to
394 assess the predictive ability of these factors in prediction models for adverse pregnancy
395 outcome.

397 **Acknowledgements**

398 We acknowledge the help of the IT department at Birmingham Women’s Hospital NHS
399 Foundation Trust with obtaining the maternity outcome data.

400 **Contribution to authorship**

401 RKM, MDK, AB designed the study, collected, analysed and interpreted the data. IM was
402 responsible for collecting the biochemistry data and in the interpretation of other results.
403 RDR and DB designed the analysis, performed the analysis and interpreted the data. All
404 authors were involved in the writing of the manuscript.

406 **Ethics approval**

407 The study had ethical approval from the research ethics committee (REC reference
408 14/NW/1394) and Confidentiality Advisory Group (CAG reference 14/CAG/1033)

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Legend of tables and figures

Table 1: Mother and fetus demographics and clinical features at the test

Table 2: Number of events for each outcome in singleton pregnancies (N=12,592) and association with PAPP-A, nuchal translucency and crown rump length (unadjusted logistic regression)

Table 3: Adjusted odds ratio estimates for the association between each adverse outcome and PAPP-A, nuchal translucency and crown rump length (adjusted logistic regression)

Legend of Supporting Information

Table S1: PAPP-A: Results from adjusted logistic regression for SGA (N=12300, M=35 imputed datasets).

Table S2: PAPP-A: Results from adjusted logistic regression for preterm birth (<37 weeks gestation) (N=12,454, M=35 imputed datasets).

Table S3: PAPP-A: Results from adjusted logistic regression for pre-eclampsia (N=12,322, M=35 imputed datasets).

Table S4: PAPP-A: Results from adjusted logistic regression for miscarriage (<24 weeks gestation) (N=10404, M=35 imputed datasets).

Table S5: PAPP-A: Results from adjusted logistic regression for stillbirth (>24 weeks gestation) (N=9753, M=35 imputed datasets)

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499 Table S6: PAPP-A: Results from adjusted logistic regression for perinatal death (death
500 between 24 weeks gestation and 7 days after birth) (N=10898, M=35 imputed datasets).

501 Table S7: PAPP-A: Results from adjusted logistic regression for neonatal death (death
502 between birth and 28 days after birth) (N=10876, M=35 imputed datasets).

503 Table S8: Nuchal translucency: Results from adjusted logistic regression for SGA (N=12299,
504 M=35 imputed datasets).

505 Table S9: Nuchal translucency: Results from adjusted logistic regression for preterm birth
506 (N=12453, M=35 imputed datasets).

507 Table S10: Nuchal translucency: Results from adjusted logistic regression for pre-eclampsia
508 (N=12321, M=35 imputed datasets).

509 Table S11: Nuchal translucency: Results from adjusted logistic regression for miscarriage
510 (N=10404, M=35 imputed datasets)

511 Table S12: Nuchal translucency: Results from adjusted logistic regression for stillbirth
512 (N=9753, M=35 imputed datasets).

513 Table S13: Nuchal translucency: Results from adjusted logistic regression for perinatal death
514 (N=10898, M=35 imputed datasets).

515 Table S14: Nuchal translucency: Results from adjusted logistic regression for neonatal death
516 (N=10876, M=35 imputed datasets).

517 Table S15: Crown rump length: Results from adjusted logistic regression for SGA (N=12299,
518 M=35 imputed datasets).

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36 531 Table S22: PAPP-A, NT and CRL: Results from adjusted logistic regression for SGA (N=12299,
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540 (N=9753, M=35 imputed datasets).

541 Table S27: PAPP-A, NT and CRL: Results from adjusted logistic regression for perinatal death
542 (N=10898, M=35 imputed datasets).

543 Table S28: PAPP-A, NT and CRL: Results from adjusted logistic regression for neonatal death
544 (N=10876, M=35 imputed datasets).

545 Table S29: PAPP-A, NT and CRL: Results from adjusted logistic regression for SGA assessing
546 functional form of PAPP-A, NT and CRL (N=12299, M=35 imputed datasets).

547 Table S30: PAPP-A, NT and CRL: Results from adjusted logistic regression for preterm birth
548 assessing functional form of PAPP-A, NT and CRL (N=12453, M=35 imputed datasets).

549 Table S31: PAPP-A, NT and CRL: Results from adjusted logistic regression for pre-eclampsia
550 assessing functional form of PAPP-A, NT and CRL (N=12321, M=35 imputed datasets).

551 **Table 1: Mother and fetus demographics and clinical features at the test.**

Summary (N=12,592)	
Mother's age (years)	30.6 (5.6)
Gestational age at test (days)	88.2 (4.3)
Parity (number)	1 [0,1]
BMI	25.1 [22.4, 28.8]
Deprivation score*, n (%)	≤8.49 455 (3.6)
	8.5 – 13.79 846 (6.7)
	13.8 – 21.35 2629 (20.9)
	21.36 – 34.17 2992 (23.8)
	≥34.18 5495 (43.6)
	Missing 175 (1.4)
Ethnicity, n (%)	African-Caribbean 944 (7.5)
	South-Asian 2502 (19.9)
	Oriental 358 (2.8)
	Other mixed 898 (7.1)
	White 7879 (62.6)
	Not stated 11 (0.1)
Assisted conception, n (%)	250 (2.0)
Smoking status, n (%)	Smoker 1569 (12.4)
	Non-smoker 10611 (84.3)
	Stopped during pregnancy 412 (3.3)
Pre-pregnancy insulin-dependent Diabetes mellitus, n (%)	36 (0.3)
Gender of baby, n (%)	Male 6118 (48.6)
	Female 6435 (51.1)
	Missing 39 (0.3)

552 Mean (standard deviation) or median [interquartile range] for continuous variables and n (%) for
553 categorical variables; * Deprivation score calculated using the National Perinatal Epidemiology Unit
554 Index of Multiple Deprivation (NPEU IMD) calculator. BMI: body mass index

Table 2: Number of events for each outcome in singleton pregnancies (N=12,592) and unadjusted association with PAPP-A, nuchal translucency and crown rump length and each outcome (univariable logistic regression).

Outcome	Number of events (%)	PAPP-A (U/L) Odds ratio (95% CI), p-value	Nuchal translucency (mm) Odds ratio (95% CI), p-value	Crown rump length (mm) Odds ratio (95% CI), p-value
SGA (<10 th customised centile)	1824 (14.5)	0.87 (0.85 to 0.90), <0.0001	0.81 (0.72 to 0.91), <0.0001	0.99 (0.99 to 1.00), 0.065
Pre-term birth (<37 weeks)	852 (6.77)	0.93 (0.90 to 0.97), <0.0001	1.15 (1.00 to 1.32), 0.053	1.00 (0.99 to 1.01), 0.730
Pre-eclampsia	352 (2.80)	0.92 (0.86 to 0.97), 0.004	0.90 (0.70 to 1.14), 0.378	1.01 (1.00 to 1.02), 0.123
Miscarriage (death prior to birth <24 weeks gestation)	73 (0.58)	0.97 (0.86 to 1.09), 0.598	1.94 (1.54 to 2.45), <0.0001	1.02 (0.99 to 1.05), 0.147
Stillbirth (death prior to birth >24 weeks gestation)	37 (0.29)	0.81 (0.66 to 1.00), 0.052	0.77 (0.35 to 1.68), 0.503	0.97 (0.93 to 1.01), 0.150
Perinatal death* (Death between 24 weeks gestation and 7 days after birth)	73 (0.58)	0.93 (0.82 to 1.05), 0.245	0.70 (0.39 to 1.23), 0.213	0.98 (0.95 to 1.01), 0.160
Neonatal death [§] (Death between birth and 28 days)	38 (0.31)	1.03 (0.89 to 1.20), 0.686	0.57 (0.25 to 1.29), 0.176	0.98 (0.95 to 1.02), 0.432

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5 557 * Perinatal death includes stillbirths; [§] neonatal death includes babies that die between birth and 7 days after birth that are captured within the perinatal
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7 558 death category. SGA small for gestational age (birth weight<10th customised centile. Odds ratios indicate the effect of 1 –unit increase in the factor on the
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9 559 odds of the outcome.
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Table 3: Adjusted odds ratio estimates for the association between each adverse outcome and PAPP-A, nuchal translucency and crown rump length.

Outcome	PAPP-A (U/L) OR (95% CI), p-value	NT (mm) OR (95% CI), p-value	CRL (mm) OR (95% CI), p-value
SGA (<10 th customised centile)	0.88 (0.85 to 0.91), <0.0001	0.79 (0.70 to 0.89), <0.0001	0.99 (0.99 to 1.00), 0.057
Preterm birth (<37 weeks)	0.92 (0.88 to 0.97), <0.0001	1.09 (0.93 to 1.27), 0.313	1.00 (0.99 to 1.01), 0.624
Pre-eclampsia toxaemia	0.91 (0.85 to 0.97), 0.003	0.87 (0.67 to 1.12), 0.287	1.01 (0.99 to 1.02), 0.325
Miscarriage (death <24 weeks gestation)	1.01 (0.84 to 1.21), 0.929	1.75 (1.12 to 2.72), 0.013	1.03 (0.99 to 1.07), 0.129
Stillbirth (death >24 weeks gestation)	0.71 (0.52 to 0.98), 0.038	0.69 (0.25 to 1.93), 0.485	0.94 (0.89 to 0.99), 0.027
Perinatal death (death between 24 weeks gestation and 7 days after birth)	0.88 (0.73 to 1.06), 0.173	0.89 (0.43 to 1.83), 0.746	0.97 (0.93 to 1.01), 0.110
Neonatal death (death between birth and 28 days)	1.04 (0.85 to 1.29), 0.687	1.07 (0.39 to 2.93), 0.896	1.00 (0.95 to 1.05), 0.919

OR odds ratio; CI confidence interval; NT nuchal translucency; CRL crown rump length; SGA small for gestational age; all odds ratio estimates for PAPP-A, NT and CRL from separate multivariable models, adjusted for maternal age, BMI, parity, ethnicity, deprivation score, smoking status, IVF, and gestational diabetes.